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Dynamics of stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) in different tissues of *Crassostrea gigas* at two contrasted ecosystems: insights from growth and food sources

Running title: Growth and isotope dynamics of oyster tissues: a matter of food

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Abstract

We studied the influence of food availability on the growth (whole body and organs) of the oyster *Crassostrea gigas* and on the dynamics of their $d^{13}C$ and $d^{15}N$ respective values. Juvenile oysters originating from Arcachon Bay were transplanted to two contrasting ecosystems, Baie des Veys (BDV) and Rade de Brest (RDB), for a 1-yr growth survey. In BDV, chlorophyll-*a* concentrations ([Chl-a]) was 3 times higher than in RDB on average, which accounts for the differences in oyster growth between BDV and RDB. Differences in trophic conditions could also explain the differences in C/N ratios and $\delta^{13}C$ values between sites; these differences widened when lipid normalised $\delta^{13}C$ values were used. Oysters clearly selected microalgae as the main food source, and especially phytoplankton. Gi (Gills), Mu (Muscle) and Re (remaining tissues) clearly exhibited different isotopic enrichment levels, with $d_{Mu} > d_{Gi} > d_{Re}$ regardless of C or N elements, culture sites and seasons. Isotopic discrimination between organs was rather equivalent between sites. This unexpected result would benefit from an experiment under condtions along a calibration of the lipid normalisation to correct $\delta^{13}C$ values in bivalves.

Keywords: food availability, isotopic discrimination, suspended particulate organic matter, oyster, bivalves, marine coastal intertidal areas, trophic ecology

1. Introduction

Suspension feeding bivalves are a key ecological component of marine food webs in coastal ecosystems (Gili and Coma, 1998; Jennings and Warr, 2003). Their filtration and biodeposition activities actively contribute to the transfer and recycling of particulate and enhance pelagic-benthic coupling (Doering et al., 1986). Most generalist feeders (*i.e.* those feeding on a mixture of different food sources according to their bioavailability) occupy an intermediate trophic niche between primary producers and secondary consumers (Lefebvre et al., 2009b). A major part of their diet is composed of microalgae, *i.e.* phytoplankton and/or resuspended microphytobenthos (also known as benthic microalgae) species (Kang et al., 2006; Yokoyama et al., 2005). Because suspension-feeding bivalves are sensitive to environmental fluctuations, such as anthropogenic impact (Piola et al., 2006), meteorological conditions (Grangeré et al., 2009), temperature (Laing, 2000), food quantity (Chauvaud et al., 2001), quality (Chaparro et al., 2008) and diversity (Riera, 2007), they act as ecological indicators of the trophic state of coastal ecosystems (Cloern and Jassby, 2008; Lefebvre et al., 2009a).

To understand the trophic role and the place of suspension feeding bivalves in ecosystems, temporal and spatial variations of the stable isotope ratios, $\delta^{13}C$ and $\delta^{15}N$, have been extensively used over recent decades (Boecklen et al., 2011). Utilization of stable isotope ratios in trophic studies is based on the observation that the isotope ratios of a consumer's tissues resemble those of its diet, with enrichment in ^{13}C and ^{15}N (DeNiro and Epstein, 1978, 1981). A common use of stable isotope ratios is to make inferences about diet from isotope values measured in tissues and food sources of a consumer (Phillips and Gregg, 2003). Numerous studies have shown that suspended particulate organic matter (SPOM) -mainly composed of phytoplankton, microphytobenthos (MPB), macroalgal detritus, riverine particulate organic matter (rPOM), bacteria, *etc*... can contribute to the diet of benthic

suspension feeders (*e.g.* Dang et al., 2009; Decottignies et al., 2007; Kang et al., 1999; Marín Leal et al., 2008; Riera and Richard, 1996). However, the main food source in most coastal marine ecosystem is microalgae such as diatoms (*e.g.* Pernet et al., 2012). The growth of suspension-feeding bivalves relies mainly on phytoplankton (PHY) during spring blooms and on other sources such as MPB outside these periods (Lefebvre et al., 2009b). Relationships between food availability to organisms and their physiological performances have thus been established for bivalve species. Kang et al. (2006) showed the importance of the seasonal development of microphytobenthos as a food source during the critical period of growth and gonad development for intertidal suspension- and deposit-feeders *Laternula marilina* and *Moerella rutila*. Sauriau and Kang (2000) showed that around 70 % of the annual production of intertidal cockles (*Cerastoderma edule*) in Marennes-Oléron Bay relied on microphytobenthos.

The dynamics of $\delta^{13}C$ and $\delta^{15}N$ values of the organs of an individual and its food sources provide valuable information on pathways of matter in its tissues. Studies by Lorrain et al. (2002), Deudero et al. (2009), Malet et al. (2007) and Paulet et al. (2006) used the seasonal variations of the $\delta^{13}C$ and $\delta^{15}N$ values in different organs of bivalves to make a detailed examination of energy allocation processes. Lorrain et al. (2002) showed that, for *Pecten maximus*, the seasonal variations in the available SPOM and its $\delta^{13}C$ and $\delta^{15}N$ values correlated with the seasonal $\delta^{13}C$ and $\delta^{15}N$ values of the scallop adductor muscle, gonad and digestive gland. These authors also found that the isotopic discrimination and turnover rates were different between organs and used this property to investigate energy and nutrient flow among them. Malet et al. (2007) used the same approach to explain physiological differences between diploid and triploid oysters (*C. gigas*). By means of a diet-switching experiment conducted in different seasons, Paulet et al. (2006) measured the isotope ratios of two suspension feeders, *P. maximus* and *C. gigas* in the gonad, the adductor muscle and the

digestive gland. They showed differences in isotope incorporation and discrimination between organs, seasons and species that reflected differences in energy allocation strategies. The transplantation experiment of *Mytilus galloprovincialis* carried out by Deudero et al. (2009) at a large spatial scale also showed different isotopic enrichment in the digestive gland, the adductor muscle and the gills due to their different turnover rates and biochemical composition.

The influence of food availability on marine bivalve growth as well as the contribution of different food sources to the diet of shellfish are widely documented in the literature. However, the seasonal dynamics of $\delta^{13}C$ and $\delta^{15}N$ values in differents tissues of the oyster *C. gigas* in its natural environment has been rarely documented (but see Malet et al., 2007). A particular attention was paid on the influence of growth and physiological state (C/N ratio) on the isotopic discrimation between tissues (muscle, gills and remaining tissues *i.e.* mostly digestive gland and mantle). To provide constrated growth conditions, 7-month old oysters were transplanted in two ecosystems where food sources differ in quantity.

2. Materials and methods

2.1. Study sites

The ecosystems of Baie des Veys (BDV, Normandie) and Rade de Brest (RDB, Bretagne), that differ in their morphodynamic and hydrobiological characteristics and in the rearing performances of *C. gigas* (Fleury et al., 2005a,b) were studied (Fig. 1). BDV, which is located in the southwestern part of the Baie de Seine, is a macrotidal estuarine system with an intertidal area of 37 km^2, a maximum tidal amplitude of *ca.* 8 m and a mean depth of *ca.* 5m. BDV is influenced by four rivers (watershed of 3500 km^2) that are connected to the bay by the Carentan and Isigny channels. In BDV, the culture site of Grandcamp (49°23’124”N, 1°05’466”W) is located in the eastern part of the bay and is characterized by muddy sand

bottoms. RDB is a 180 km^2 semi-enclosed marine ecosystem connected to the Iroise Sea by a deep narrow strait. Half of its surface area is less than 5 m in depth (mean depth = 8m). Five rivers flow into RDB, but two of these account for 50 % of the freshwater inputs: the Aulne in the south (watershed of 1842 km^2) and the Elorn in the north (watershed of 402 km^2). In RDB, the study site at Pointe du Château (48°20'03"N, 04°19'145"W) is located at the mouth of the Daoulas river in an area with gravel and rubble bottoms that likely prevent a high development and re-suspension of microphytobenthos species.

2.2. Environmental data: chlorophyll-*a* and temperature

Chlorophyll-*a* concentration ([Chl-*a*], $\mu g.L^{-1}$) data were provided by the Ifremer national REPHY network for phytoplankton monitoring (http://wwz.ifremer.fr/lerpc/Activites-et-Missions/Surveillance/REPHY), at Géfosse in BDV (49°23'47"N, 1°06'36"W, *i.e.* at a distance of 1.5 km from the culture site) and Lanvéoc in RDB (48°18'33.1"N, 04°27'30.1"W, *i.e.* at a distance of 10.5 km from the culture site). [Chl-*a*] were obtained after filtering samples of seawater and determined following Aminot & Kerouel (2004). Water samples were collected at high spring tide (Fig. 1). At each site, seawater temperature was measured continuously (high-frequency recording) using a multiparameter probe (a Hydrolab DS5-X OTT probe in RDB and a TPS NKE probe in BDV).

2.3. Sample collection and analysis

2.3.1. Oysters

Juveniles of the oyster *C. gigas* (mean shell length= 2.72 cm±0.48; mean dry flesh mass= 0.020 g±0.008) originating from Arcachon Bay were split into two groups and transplanted to the two culture sites in March 2009. Oysters were reared from March 2009 to February 2010 in plastic net culture bags attached to iron rearing tables at 60 cm above the bottom. The

rearing tables were placed in the intertidal area, so that the oysters were subjected to tidal action, *i.e.* immersed *ca.* 70 % of the day (average daily immersion time over a year). Samples were taken every two months during autumn and winter, and monthly during spring and summer. At each sampling date, 30 oysters (with individual mass representative of the mean population mass) were collected in the two sites. They were cleaned of epibiota and maintained alive overnight in filtered seawater to evacuate their gut contents. The oysters were individually measured (shell length), opened for tissue dissection and carefully cleaned with distilled water to remove any shell debris. After dissection, the tissues were frozen (-20°C), freeze-dried (48 h), weighed (total dry flesh mass, DW, g), ground to a homogeneous powder and finally stored in safe light and humidity conditions for later isotopic analyses. From March until late June 2009, five individuals out of the 30 sampled were randomly selected for whole body stable isotope analyses. From July 2009, the gills (Gi) and the adductor muscle (Mu) of the five oysters were dissected separately from the remaining tissues (Re), *i.e.* mantle, gonad, digestive gland and labial palps. As for the whole body tissues, Gi, Mu and Re were frozen at -20°C, freeze-dried (48 h) and weighed (DW_{Gi}, DW_{Mu} and DW_{Re}) prior to being powdered and stored until stable isotope analyses. From July 2009 the total dry flesh mass was then estimated as: $DW = DW_{Gi} + DW_{Mu} + DW_{Re}$.

2.3.2. Organic matter food sources (OMS)

In BDV and RDB, suspended particulate organic matter (SPOM) was sampled for stable isotope analyses in the surroundings of each culture site at high tide. Two replicate samples of seawater were taken in 2 L bottles at subsurface (0-50 cm), and then filtered onto preweighed, pre-combusted (450°C, 4 h) Whatmann GF/C (Ø= 47 mm) glass fibre filters immediately after sampling and pre-filtering onto a 200 µm mesh to remove the largest particles. In BDV, microphytobenthos (MPB), which can be re-suspended from the sediment by waves and tidal

action and contributes to the diet of *C. gigas* (Marín Leal et al., 2008) was also sampled. MPB was collected by scraping the visible microalgal mats off of the sediment surface adjacent to the culture site during low tide. Immediately after scraping, MPB and sediment were put into sea water where they were kept until the extraction at the laboratory. MPB was extracted from the sediment using Whatmann lens cleaning tissue (dimensions: 100 ×150 mm, thickness: 0.035 mm). The sediment was spread in a small tank and covered with two layers of tissue. The tank was kept under natural light/dark conditions until migration of the MPB. The upper layer was then taken and put into filtered sea water to resuspend the benthic microalgae. The water samples were then filtered onto pre-weighed, precombusted (450°C, 4 h) Whatmann GF/C (Ø = 47 mm) glass-fibre filters. Meiobenthic fauna (mainly nematodes) was removed from the filters under a binocular microscope. No samples of MPB were collected in RDB due to the bottom composition of the culture site (mostly gravels). The SPOM and MPB filters were then treated with concentrated HCl fumes (4 h) in order to remove carbonates (according to Lorrain et al., 2003), frozen (-20°C) and freeze-dried (60°C, 12 h). Both the filters were then ground to a powder using a mortar and pestle and stored in safe light and humidity conditions until isotope analyses. Phytoplankton dominated SPOM isotope values were retrieved from Lefebvre et al. (2009b) for BDV ($SPOM_{phyBDV}$), and from the French Coastal Monitoring Network SOMLIT (Service d'Observation en Milieu LITtoral, http://somlit.epoc.u-bordeaux1.fr/fr/, Brest Portzic "BreP" station) for RDB ($SPOM_{phyRDB}$). Finally, isotope values for epipelic MPB in BDV (MPB_{epiBDV}) were also taken from Lefebvre et al. (2009b).

2.4. Elemental and stable isotope analyses

The samples of oyster tissues and OMS were analysed using a CHN elemental analyser EA3000 (EuroVector, Milan, Italy) for particulate organic carbon (POC) and particulate

nitrogen (PN) in order to calculate their C/N atomic ratios (Cat/Nat). Analytical precision for the experimental procedure was estimated to be less than 2 % dry mass for POC and less than 6 % dry mass for PN. The gas resulting from the elemental analyses was introduced online into an isotopic ratio mass spectrometer (IRMS) IsoPrime (Elementar, UK) to determine the $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios. Isotopic ratios are expressed as the difference between the samples and the conventional Pee Dee Belemnite (PDB) standard for carbon, and air N_2 for nitrogen, according to the following equation:

$$\delta^0_{ij} = \left(\frac{R_{sample}}{R_{standard}} - 1\right) 1000 \quad (1)$$

where δ^0_{ij} (‰) is the isotope 0 (13 or 15) of element i (C or N) in a compound *j*. Subscript *j* stands for the whole soft tissues DW, the gills Gi, the adductor muscle Mu, the remaining tissues Re of *C. gigas* or the food sources SPOM and MPB. R is the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio. The standard values of R are 0.0036735 for nitrogen and 0.0112372 for carbon. When the organs were sampled, the isotopic ratio and the C/N ratio of the whole soft tissues, i.e. δ^0_{iDW} and C/N_{DW} , respectively, were calculated as follows:

$$\delta^0_{iDW} = \frac{\delta^0_{iGi} DW_{Gi} + \delta^0_{iMu} DW_{Mu} + \delta^0_{iRe} DW_{Re}}{DW_{Gi} + DW_{Mu} + DW_{Re}} \quad (2)$$

$$C/N_{DW} = \frac{C/N_{Gi} DW_{Gi} + C/N_{Mu} DW_{Mu} + C/N_{Re} DW_{Re}}{DW_{Gi} + DW_{Mu} + DW_{Re}} \quad (3)$$

The internal standard was the USGS 40 of the International Atomic Energy Agency ($\delta^{13}C$ = -26.2 ‰; $\delta^{15}N$ = -4.5 ‰). The typical precision in the analyses was ±0.05 % for C and ±0.19 % for N. One tin caps per sample was analysed. The mean value of the isotopic ratio was considered for both animal tissues and OMS.

2.5. Statistical analyses

Firstly, comparisons of growth patterns and isotopic composition of whole body tissues between the BDV and RDB sites were based on the average individual dry flesh mass (DW), the $\delta^{13}C$ and $\delta^{15}N$ values and the C/N ratio. Differences among DW, $\delta^{13}C$ and $\delta^{15}N$ values and C/N ratio were analysed by a two-way ANOVA, with time (*i.e.* sampling date) and site as fixed factors. Secondly, repeated measures ANOVAs were used to test for differences in the individual dry mass, isotopic ratios and C/N ratio among the different organs (gills, adductor muscle and remaining tissues) of *C. gigas*, with site and sampling date as the (inter-individual) sources of variation among oysters, and organs as the (intra-individual) source of variation within oysters. In both cases, all data were square-root transformed to meet the assumptions of normality, and the homogeneity of variance and/or the sphericity assumption were checked. In cases where ANOVA results were significant, they were followed by a Tukey HSD post-hoc test (Zar, 1996) to detect any significant differences in dry mass, isotopic ratios and C/N ratio for the whole body and for each of the organs between the two sites and/or among the different organs.

2.6. Correction of isotopic values

Isotopic values were corrected for two purposes. First, the mean observed C/N ratios were found higher than 3.5 (except adductor muscle), above this value lipid normalisation is recommended (Post et al., 2007). Normalisation of $\delta^{13}C$ values was performed according to: $\delta^{13}C_{normalised} = \delta^{13}C_{untreated} - 3.32 + 0.99$ C:N (Post et al., 2007). Secondly, determination of the diet was based on visual inspection of the biplot by further correcting $\delta^{13}C$ and $\delta^{15}N$ values with the trophic enrichment factor (or trophic discrimination Δ) as measured for *C. gigas* by Dubois et al.(2007) during a diet-switch experiment: $\Delta\delta^{13}C = 1.9$ ‰ and $\Delta\delta^{15}N = 3.8$ ‰.

3. Results

3.1. Abiotic conditions and food availability

From March 2009 to February 2010, [Chl-*a*] was on average three times higher in BDV than in RDB, with maximum values of 9.1 $\mu g.L^{-1}$ in June 2009 in BDV and 4.4 $\mu g.L^{-1}$ in May 2009 in RDB (Fig. 2). From March to July 2009, the average [Chl-*a*] was 2.3 $\mu g.L^{-1}$ in RDB against 4.9 $\mu g.L^{-1}$ in BDV, *i.e.* which was relatively high compared with the average [Chl-*a*] values of 0.5 $\mu g.L^{-1}$ in RDB and of 1.4 $\mu g.L^{-1}$ in BDV from October 2009 to February 2010 (Fig. 2). Seawater temperature in RDB and BDV showed a typical seasonal pattern with some similarities among the 2 sites, *i.e.* increasing values between March and August 2009, up to a maximum value in July or August 2009, followed by a decrease during the autumn (Fig. 2). The thermal amplitude was, however, slightly higher in BDV (15.3°C) than in RDB (13.7°C). In BDV, the maximum and minimum temperatures were reached in August 2009 (20.8°C) and March 2010 (6.6°C) respectively, while in RDB they occurred in early July 2009 (19.7°C) and January 2010 (4.4°C), respectively.

3.2. Growth and elemental ratios of *C. gigas* tissues

The mass gain of oysters expressed in total dry flesh mass DW differed significantly over time between the two ecosystems RDB and BDV (2-way ANOVA, site × time, $F_{7,455} = 40.79$, $P < 0.0001$; Fig. 3 A). From March to June 2009, the increase in DW was relatively slow and similar in BDV and RDB (from 0.02 g to 0.36 g in BDV and from 0.02 g to 0.39 g in RDB), with no significant differences between sites at any sampling date (Tukey HSD post-hoc test, $0.194 \leq P \leq 0.513$). From July 2009, DW was significantly higher in BDV than in RDB at each sampling date (Tukey HSD post-hoc test, $P < 0.0001$). DW increased more sharply from July until October 2009 in BDV compared with RDB where growth ceased in August and where a slight decrease in DW was observed until February 2010 (Fig. 3A). At the end of the survey (February 2010), the value for DW in BDV was 1.80 g compared with 0.55 g in RDB,

which respectively corresponds to a mass gain of *ca.* 8 times and 2.5 times from the transplantation in March 2009.

Similarly to DW, significant interactions between site, time and organs were noted for the dry mass of the different organs, DW_{Gi}, DW_{Mu}, and DW_{Re}, indicating that the mass gain varied over time differently among the organs between the two ecosystems (3-way ANOVA, $F_{8,307}$ = 8.14, $P < 0.0001$, Fig. 3 C&E). In BDV, DW_{Re} showed an increase of *ca.* 30 % between July and October 2009, whereas it decreased by *ca.* 12 % over the same period in RDB (Fig. 3 C). At both sites, DW_{Re} was stable from November 2009 until February 2010 (Fig. 3 C&E). Between August 2009 and February 2010, 70 % and 80 % of DW corresponded to DW_{Re} in RDB and BDV respectively. From July 2009 to February 2010, DW_{Mu} and DW_{Re} were significantly different between the two sites at each sampling date (Tukey HSD post-hoc test, $P \leq 0.0346$), while DW_{Gi} was not significantly different between BDV and RDB at any sampling date between July and October 2009 (Tukey HSD post-hoc test, $0.0541 \leq P \leq 0.1550$). In BDV, DW_{Gi} and DW_{Mu} did not differ significantly in July 2009 (Tukey HSD post-hoc test, $P = 0.0682$; Fig. 3C); in RDB, they were also no significant differences in July, August, October 2009 or in February 2010 (Tukey HSD post-hoc test, $0.0970 \leq P \leq 0.9134$, Fig. 3E).

The C/N ratio of whole soft body tissues (C/N_{DW}) was significantly higher in BDV than in RDB at almost all sampling dates (2-way ANOVA, site × time $F_{7,78} = 2.96$, $P = 0.0096$, Fig. 3B). Only in June 2009 C/N_{DW} did differ significantly between BDV and RDB (Tukey HSD post-hoc test, $P = 0.1667$). In BDV, a strong increase of *ca.* 87% was observed from April to August 2009, when C/N_{DW} reached the maximum value of 6.9. At the same time, the C/N_{DW} ratio remained rather constant in RDB, with a mean value of 4.3 over the whole survey period. In BDV, C/N_{DW} in BDV fell to the value of 5.8 in February 2010 (Fig. 3B). C/N ratio of oyster organs C/N_{Re}, C/N_{Gi} and C/N_{Mu} changed over time in a different manner between

ecosystems as shown by significant interactions between site, time and organs (3-way ANOVA, $F_{8,44} = 4.21$, $P = 0.0008$, Fig. 3 D & F). C/N_{Re} showed similar variations to C/N_{DW} (Fig. 3 B, D & F). C/N_{Re}, C/N_{Gi} and C/N_{Mu} were significantly different from one another in BDV and RDB at all sampling dates (Tukey HSD post-hoc test, $P \leq 0.0372$) and ranked as follows: $C/N_{Re} > C/N_{Gi} > C/N_{Mu}$ irrespective of the study site.

3.3. Stable isotope ratios of *C. gigas* whole body

Both $\delta^{13}C_{DW}$ and $\delta^{15}N_{DW}$ of *C. gigas* (whole body) over time differed significantly between BDV and RDB ecosystems (2-way ANOVAs, site × time, $F_{7,78} = 31.82$, $P < 0.0001$ for $\delta^{13}C_{DW}$ and $F_{7,78} = 11.23$, $P < 0.0001$ $\delta^{15}N_{DW}$, Fig. 4A&B). With the exception of February 2010 ($P=0.9568$; Fig. 4A), $\delta^{13}C_{DW}$ was significantly lower in BDV than in RDB at all other sampling dates (Tukey HSD post-hoc test, $P <0.05$), with a mean $\delta^{13}C_{DW}$ of -20.6 ‰ in BDV and -19.5 ‰ in RDB. From March to May 2009, $\delta^{13}C_{DW}$ in RDB decreased from -19.3 ‰ to -20.6 ‰ and then increased up to -18.9 ‰ in July 2009. The highest $\delta^{13}C_{DW}$ value, -18.8 ‰, was reached in August 2009 and a slight decrease was then observed until the end of the survey in RDB. A sharp decrease in $\delta^{13}C_{DW}$ occurred in BDV from -19.3 ‰ in March 2009 to -22.1 ‰ in May 2009 (Fig. 4A). Between June and July 2009, $\delta^{13}C_{DW}$ leapt up to the value of -19.9 ‰ and remained constant until February 2010. When normalising for lipids, $\delta^{13}C_{DW}$ values followed approximately the same pattern in BDV and RDB with slightly higher values in BDV from October 2009. Except in June 2009 when no significant differences were observed (Tukey HSD post-hoc test, $P = 0.8610$), $\delta^{15}N_{DW}$ was significantly higher in BDV than in RDB ($P \leq 0.0143$; Fig. 4B). The maximum values for $\delta^{15}N_{DW}$ were 9.5 ‰ in August 2009 and 10.3 ‰ in September 2009 in RDB and in BDV, respectively.

3.4. Stable isotope ratios of *C. gigas* organs and isotopic discrimination

Temporal variations in $\delta^{13}C$ and in $\delta^{15}N$ of the different organs showed similar patterns to $\delta^{13}C_{DW}$ and $\delta^{15}N_{DW}$ at both sites (Fig. 4). The interactions between site, time and organ were significant for $\delta^{13}C$ and $\delta^{15}N$ values, indicating that stable isotope ratios of oyster organs changed over time in a different way between ecosystems (3-way ANOVA, $F_{8,48} = 8.88$, $P < 0.0001$ and $F_{8,48} = 8.88$, $P = 0.0002$ respectively, Fig. 4 C, D, E & F).

From August 2009 to February 2010, $\delta^{13}C_{Gi}$, $\delta^{13}C_{Mu}$ and $\delta^{13}C_{Re}$ values (*i.e.* isotope ratios for the gills, the adductor muscle and the remaining tissues) values decreased for oysters in RDB, while they remained stable in oysters at BDV (Fig. 4 C & E). No significant differences were observed between BDV and RDB in October and November 2009 for the $\delta^{13}C_{Gi}$ (Tukey HSD post-hoc test, $P = 0.1037$ and $P = 0.1345$, respectively), in November 2009 and February 2010 for the $\delta^{13}C_{Re}$ (Tukey HSD post-hoc test, $P = 0.0729$ and $P = 0.6928$, respectively) and in February 2010 for the $\delta^{13}C_{Mu}$ (Tukey HSD post-hoc test, $P = 0.1279$). In October 2009, the $\delta^{13}C_{Gi}$ and the $\delta^{13}C_{Mu}$ values did not differ significantly from each other in BDV (Tukey HSD post-hoc test, $P = 0.8190$). Significant differences among $\delta^{13}C_{Gi}$, $\delta^{13}C_{Mu}$ and $\delta^{13}C_{Re}$ were found in BDV and in RDB (Tukey HSD post-hoc test, $P \leq 0.0371$) at all other sampling dates (Fig. 4 C&E).

$\delta^{15}N_{Gi}$, $\delta^{15}N_{Mu}$ and $\delta^{15}N_{Re}$ were significantly different between the two sites at most dates (Tukey HSD post-hoc test, $P \leq 0.0064$), except in July 2009 for $\delta^{15}N_{Re}$ (Tukey HSD post-hoc test, $P = 0.1041$; Fig. 4 D&F). Values were rather constant in RDB over the whole survey, while they increased from June to September 2009 in BDV and then decreased slightly and stabilised until February 2010 (Fig. 4 C&E). The $\delta^{15}N_{Gi}$, $\delta^{15}N_{Mu}$ and $\delta^{15}N_{Re}$ were significantly different from one another within BDV and RDB (Tukey HSD post-hoc test, $P \leq 0.0371$) at all sampling dates.

At the two sites, $\delta^{13}C$ and $\delta^{15}N$ values ranked in the same order: $\delta^{13}C_{Mu} > \delta^{13}C_{Gi} > \delta^{13}C_{Re}$ and $\delta^{15}N_{Mu} > \delta^{15}N_{Gi} > \delta^{15}N_{Re}$ (Fig. 4 C,D,E & F). The isotopic discrimination between $\delta^{13}C$ and $\delta^{15}N$ values for organs (noted $\Delta\delta^{13}C$ and $\Delta\delta^{15}N$ respectively) were very close for the two sites when considering uncorrected $\delta^{13}C$ values (Fig 5A). Only for gills, $\delta^{13}C$ and $\delta^{15}N$ values were slightly higher in BDV than in RBD. When applying lipid normalization of $\delta^{13}C$ values to the organs, this difference in $\delta^{13}C_{Gi}$ and $\delta^{15}N_{Gi}$ between RDB and BDV was widened (Fig. 5B). However, $\delta^{13}C$ values after correction for lipids were significantly higher in BDV than in RDB and differed significantly compared to the scenario without any lipid normalisation (Fig. 5A & B).

3.5. Stable isotope ratios of food sources and diet of *C. gigas*

The $\delta^{13}C$ and $\delta^{15}N$ values of the suspended particulate organic matter SPOM food source (*i.e.* $\delta^{13}C_{SPOM}$ and $\delta^{15}N_{SPOM}$) exhibited different patterns in BDV and RDB (Fig. 6A and B). From May to September 2009, the $\delta^{13}C_{SPOM}$ decreased from -18.5 ‰ to -25.4 ‰ in RDB (Fig. 6A). From September 2009 to late October 2009, $\delta^{13}C_{SPOM}$ varied over a range of 2.4 ‰ and then stabilised. In BDV, a sharp increase in $\delta^{13}C_{SPOM}$ occurred in June and July 2009 when the maximum value was reached, *i.e.* $\delta^{13}C_{SPOM}$ = -18.5 ‰ followed by a decrease of around 5.2 ‰ over the next three months (Fig. 6A). $\delta^{15}N_{SPOM}$ values in RDB varied between 6.5 ‰ and 8.1 ‰ from May to September 2009 and dropped to 5.5 ‰ in October 2009 (Fig. 6B), with an average of 7.2 ‰ over the whole sampling period. In BDV, $\delta^{15}N_{SPOM}$ was 8.4 ‰ on average, but showed high temporal variability throughout the survey. The highest $\delta^{15}N_{SPOM}$ is observed in BDV in late October 2009, reaching a value of 10.1 ‰ (Fig. 6B). It should be noticed that the $\delta^{13}C_{SPOM}$ and $\delta^{15}N_{SPOM}$ values in BDV were higher by 3.8 ‰ and 3.2 ‰ respectively than the $SPOM_{phyBDV}$ values (Fig. 7). In RDB, $\delta^{13}C_{SPOM}$ and $\delta^{15}N_{SPOM}$ were also higher than the

$SPOM_{phyRDB}$ values by 0.9 ‰ and 1.2 ‰ respectively (Fig. 7). The opposite pattern was observed for the microphytobenthos food source in BDV: the $\delta^{13}C_{MPB}$ and $\delta^{15}N_{MPB}$ values in the present study were lower than the $\delta^{13}C_{MPBepi}$ and $\delta^{15}N_{MPBepi}$ values by 3.5 ‰ and 2.1 ‰, respectively (Fig. 7).

As for *C. gigas* tissues, the mean $\delta^{13}C_{DW}$ and $\delta^{15}N_{DW}$ values that were corrected for trophic discrimination and also lipid-normalized differed significantly from the values of SPOM in both BDV and RDB. For BDV, these values were close to those ones of $SPOM_{phyBDV}$. $\delta^{13}C_{DW}$ values first decreased slowly over time and then increased, while $\delta^{15}N_{DW}$ increased gradually. Except for the first two points which are below the range of $SPOM_{phyBDV}$, $SPOM_{phyBDV}$ values scatter in a crescent shape plot towards the MPB values (Fig. 7). For RDB, the corrected values of $\delta^{13}C_{DW}$ equaled the $SPOM_{phyRDB}$ values. In parallel, $\delta^{15}N_{DW}$ increased gradually over time, the first value being *ca*. 3 ‰ below $\delta^{15}N_{SPOMphyBDV}$ (Fig. 7).

4. Discussion

Oyster growth and C/N ratio vary with food availability

[Chl-*a*], as a quantitative proxy of microphyte food availability, influences oyster growth (in terms of dry flesh mass DW) differently at the sites BDV and RDB. The seasonal differences in [Chl-*a*] between BDV and RDB, which are particularly marked in spring and early summer, *i.e.* $[Chl\text{-}a]_{BDV}$ *ca.* 4 $[Chl\text{-}a]_{RDB}$, likely account for the differences in oyster growth performances observed between the two sites (Figs. 2, 4A and 5 A). An increase in DW occurs from March to June 2009 (Fig. 4A) when Chl-*a* is likely non-limiting in both BDV and RDB sites. This suggests that the growth of *C. gigas* (as expressed in DW) in BDV and in RDB mainly relies on the microphytes food source. The growth patterns of *C. gigas* in BDV slightly differ from the results of Grangeré et al. (2009) and Marín Leal et al. (2008), who reported a sharp decrease of DW in spring, due to spawning events, and in autumn and winter,

probably due to low food availability. The continuous growth of *C. gigas* in DW observed from March 2009 to February 2010 in our study can be explained by the unusual [Chl-*a*] in BDV in 2009: blooms did not exceed 9.1 $\mu g.L^{-1}$ and but stretched over four months (from May to August). Conversely, Grangeré et al. (2009), Jouenne et al. (2007) and Lefebvre et al. (2009b) reported larger blooms, between 12 $\mu g.L^{-1}$ and 25 $\mu g.L^{-1}$, earlier in the year (March and April).

Similarly to DW, the dry mass and C/N ratios of the different organs, and especially the remaining tissues Re pool, were also influenced by food availability. The high contribution of DW_{Re} to the total dry mass of oysters, *i.e* 70 % and 80 % in BDV and RDB (Fig. 3C, E), respectively, can be explained by the fact that *C. gigas* stores energy (mainly lipids and glycogen) in the digestive gland, gonad and mantle during spring and summer (*e.g.* Berthelin et al., 2000; Costil et al., 2005; Ren et al., 2003; Whyte et al., 1990). The decrease in DW_{Re} for oysters in RDB from July 2009 to March 2010 may result from the low quantity of food *i.e.* [Chl-*a*] = 0.6 $\mu g.L^{-1}$ observed from July 2009 to February 2010. The C/N ratios of the gills and the adductor muscle tend to remain constant over time, suggesting that these two organs make very little contribution to reserve storage.

SPOM isotopic value is highly variable

With respect to the composition of available food, the temporal variations of SPOM in BDV differ from the typical patterns observed in this bay. In BDV, the SPOM source was slightly higher in both $\delta^{13}C$ and $\delta^{15}N$ values, ranging from -27.2 ‰ to -21.5 ‰ and from 6.3 ‰ to 10.1 ‰, respectively, compared with previous ranges of values that have been reported, *ca.* -22 ‰ to -18 ‰ for $\delta^{13}C$ and *ca.* 3 ‰ to *ca.* 6 ‰ for $\delta^{15}N$ values in 2004 and 2005 (Lefebvre et al., 2009b; Marín Leal et al., 2008). The isotope ratios of epipelic microphytobenthos species ($\delta^{13}C_{MPBepi}$ and $\delta^{15}N_{MPBepi}$) also differed from the values usually observed in BDV

(Fig. 6). $\delta^{13}C_{MPBepi}$ was higher than previously reported (overall mean = -14.8 ‰), though the $\delta^{15}N_{MPBepi}$ were lower (overall mean = 5.5 ‰) than the MPB values found by Marín Leal et al. (2008) in 2004 and 2005 (overall mean = -17.9 ‰ and 7.4 ‰, respectively). On the contrary, these values are comparable to typical values of epipelic MPB of mudflats (Malet et al., 2008). The sampling methods of the epipelic and epipsammic fractions of microphytobenthos by Lefebvre et al. (2009b) and Marín Leal et al. (2008) may account for the differences with our values, which only refered to diatoms that had migrated through the sediment (Fig. 7). Moreover, the $\delta^{15}N_{MPBepi}$ was lower than the $\delta^{15}N_{SPOM}$ in our study, while the opposite trend was previously reported (Kang et al., 2006; Riera, 2007; Yokoyama et al., 2005). Our sampling in the immediate surroundings of the culture sites differs from Lefebvre et al. (2009b) and from Marín Leal et al. (2008), *i.e.* the seaward samples of SPOM are likely dominated by phytoplankton (PHY, Fig. 7). SPOM samples are time dynamic mixing of alive and detritus particles in the estuarine-coastal zone (Malet et al., 2008). Additionnally, isotopic ratios of alive particles can have their own temporal dynamics depending on the availability of nutrients: for example, $\delta^{13}C$ values of phytoplankton cells typically increase during blooms when dissolved CO_2 availability decreases and so does the fractionation (Savoye et al., 2003). Thus, it is very likely that the samples of our study contained more detritus from macroalgae and riverine particulate organic matter (rPOM) than MPB or PHY.

Oysters select microalgae in their diet

However, the oyster diet in the two systems mainly relied on a marine phytoplankton food source (PHY, *e.g.* Kang et al., 2006; Marín Leal et al., 2008), as indicated by the isotopic enrichment of oysters relative to the $SPOM_{phy}$ values in the two study sites (Fig. 7). The capacity for pre-ingestive particle processing has been described in *C. gigas*, resulting in an efficient selection of organic particles over inorganic ones (Barillé et al., 1997). The selection

efficiency within organic material was demonstrated, resulting in a positive selection of live cells against detritus (Ward and Shumway, 2004). This possibly explained the differences between SPOM, $SPOM_{phy}$ and oyster tissue isotope values in our study (Fig. 7). During autumn, oysters in BDV continued to grow while their $\delta^{13}C_{DW}$ increased and their $\delta^{15}N_{DW}$ decreased (trend mostly driven by $\delta^{13}C_{Re}$ and $\delta^{15}N_{Re}$), suggesting that MPB_{epi} may have contributed to the diet of *C. gigas* when PHY availability was low (Fig. 7, Marin-Leal et al., 2008). Accordingly, pelagic microphytes (mostly phytoplankton and to a less extend resuspended MPB) enumeration data have been used as a suitable trophic forcing variable to simulate the variability of growth and reproduction of *C. gigas* in RDB and BDV (Alunno-Bruscia et al., 2011, Bernard et al., 2011). The contribution of MPB to the diet of oysters that was found in BDV in our study seems rather low compared to previous results on this site (Lefebvre et al., 2009b, Marin Leal et al., 2008). According to Grangeré et al. (2012), an interannual variability in the contribution of MPB to the diet of *C. gigas* was observed over 4 years (2005 to 2008): a low contribution of MPB coincided with wet years (2007 and 2008) when phytoplankton production was higher due to higher nutrient input from rivers. With annual cumulated precipitations of *ca*. 870 mm (Météo France data), which is rather close to precipitations measured in 2007 (926 mm) and 2008 (918 mm), 2009 ranks as a wet year (compared to dry years i.e. 2005 and 2006 *ca.* 750 mm), possibly explaining the low contribution of MPB this year.

Isotopic discrimination vary slightly between sites

The ranking $\delta_{Mu} > \delta_{Gi} > \delta_{Re}$ is observed in the enrichment patterns of ^{13}C and ^{15}N between organs, irrespective of the study site. This pattern is consistent with results from previous studies. Malet et al. (2007) found that $\delta^{13}C$ and $\delta^{15}N$ were higher in the adductor muscle than in the mantle, digestive gland and gonad of diploid and triploid *C. gigas*. Paulet et al. (2006)

showed that the adductor muscle had higher $\delta^{13}C$ values than the gills and the gonad of oysters in a diet-shift experiment. In the same way, Yokoyama et al. (2005) pointed out that $\delta^{15}N_{Mu}$ in *C. gigas* was greater than $\delta^{15}N_{Gi}$ after reaching equilibrium with the food source. δ_{Re} vary faster over time than δ_{Mu} and δ_{Gi} (Fig. 4 C, D, E & F). These results suggest that uptake food is preferentially routed to the storage organs, *e.g.* digestive gland and mantle, and/or reproductive tissues during active growth periods in spring and early summer. The narrow range of variation in δ_{Mu} (and to a lesser extent of δ_{Gi}) over the survey might be due to the lower turnover rate of this organ. The carbon incorporation index calculated by Paulet et al. (2006) in the muscle of both *C. gigas* and *P. maximus* showed the lowest value among all organs studied in all seasons. In *P. maximus*, $\delta^{13}C_{Mu}$ and $\delta^{15}N_{Mu}$ show similarly low variation compared with the digestive gland and the gonad (Lorrain et al., 2002). The differences in isotopic discrimination between organs ($\Delta\delta^{13}C$) that have been reported in the literature (*e.g.* Deudero et al., 2009; Guelinckx et al., 2007; Suzuki et al., 2005; Tieszen et al., 1983) can be mainly explained by differences in the biochemical composition of the different organs. Organs containing a high proportion of lipids have a lower $\delta^{13}C$ value than organs with a lower lipid content (or a higher protein content), since lipids are depleted in ^{13}C (Post et al., 2007). The low $\delta^{13}C_{Re}$ and high C/N_{Re} ratios are thus consistent with the physiological role of these tissues, *i.e.* energy storage and reproduction, since gametogenesis in spring and summer is characterised by an increase in lipids and glycogen (Berthelin et al., 2000; Soudant et al., 1999). The relatively high values of $\delta^{13}C_{Mu}$ and $\delta^{15}N_{Mu}$, combined with a high protein concentration in the adductor muscle (low C/N ratio), supports the idea that the adductor muscle is not a storage compartment supplying the energetic needs of reproduction (Berthelin et al., 2000). The simultaneous increases of DW_{Re} and C/N_{Re} and the low values of the $\delta^{13}C_{Re}$ from February to October 2009 in BDV confirm that most of the energy assimilated during spring and summer is directed to the reserve tissues (Figs. 3D and 4C). The same pattern was

490 previously shown by Malet et al. (2007) for *C. gigas* and by Smaal and Vonk (1997) for *Mytilus edulis*. The $\delta^{13}C$ lipid normalisation after Post et al. (2007) confirms the impact of the C/N ratio on $\delta^{13}C$ values (Fig 5B), and more particularly for the remaining tissues Re in which a significant part of glycogen accounts for high C/N. However, such a relationship has never been reported for bivalves. When applying the lipid normalisation, the enrichment in
495 ^{13}C for Re compared to Gi (Fig. 5B) may have two non exclusive origins, either higher $\delta^{13}C$ values in the food that oyster ingested before sampling, or an overestimation of the lipid correction. Finally, except for the gills (Gi), the $\delta^{15}N$ isotopic discrimination in organs ($\Delta\delta^{15}N$) was very close between the two sites, thus leading to a constant $\Delta\delta^{15}N$ whatever the growth. Though surprising, this is is an interesting result since, at the whole body level,
500 trophic fractionation (or trophic enrichment) is supposed to decrease with growth (Lefebvre & Dubois, this issue) or with food level (Emmery et al., 2011). An experiment under controlled food and temperature conditions would certainly help deciphering the causes for $\Delta\delta^{13}C$ and $\Delta\delta^{15}N$ differences between organs, *e.g.* different turnover rates, food level and/or dynamic isotopic values of food sources.

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Concluding remarcks

As a conclusion, the results of our transplantation experiment showed that temporal variations in food availability (quantity, [Chl-*a*] and stable isotope ratios) and also in growth (in dry flesh mass, DW) and physiological state (C/N ratio) of *C. gigas* both influenced the dynamics
510 of stable isotopes of the whole body tissues and of the organs (gills, adductor muscle and remaining tissues) of oysters. The oyster diet was mainly composed of microalgae and especially phytoplankton confirming that *C. gigas* is an opportunistic suspension feeder that can modify its diet as a function of available food on temporal and spatial scales (Lefebvre et al., 2009b). A calibration of the lipid correction for $\delta^{13}C$ values should be done on bivalves,

taking into account the high content of glycogen in digestive gland at certain periods of the year. Finally, these results and the constant isotopic discrimation between organs would benefit to be confirmed under controlled conditions.

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680 Figure captions

Figure 1: Geographic location of the two study ecosystems, Baie des Veys (BDV) and Brest Harbour (RDB), along the Channel and Atlantic coasts of France, respectively. White circles indicate the oyster culture sites and black circles indicate the locations where chlorophyll-*a* 685 concentration [Chl-*a*] and temperature were monitored.

Figure 2: Temporal variations in chlorophyll-*a* concentration ([Chl-*a*], $\mu g.L^{-1}$, solid lines) and water temperature (Temp., °C, dashed lines) from March 2009 to March 2010 at two sites: Baie des Veys in Normandy (BDV, grey lines) and Rade de Brest in North Brittany (RDB, 690 black lines). The arrow indicates the oyster transplantation date (start of the survey).

Figure 3: Temporal variations in mean individual dry flesh mass (DW, g, left panels) and C/N ratio (right panels) of *Crassostrea gigas* tissues from March 2009 to February 2010 at two sites: Baie des Veys in Normandy (BDV, white symbols) and Rade de Brest in North Brittany 695 (RDB, black symbols). Panels A and B shows values of the whole body tissues. Panels C, D, E, F shows the values of the organs: the remaining tissues Re (including the mantle, gonad, digestive gland and labial palps), the adductor muscle Mu and the gills Gi. The vertical bars indicate ±SD of the mean for n = 30 oysters. Note that in E, Gi and Mu are surimposed.

700 Figure 4: Temporal variations in mean individual $\delta^{13}C$ (‰, left panels) and $\delta^{15}N$ (‰, right panels) values of *Crassostrea gigas* tissues from March 2009 to February 2010 at two sites: Baie des Veys in Normandy (BDV, white symbols) and Rade de Brest in North Brittany (RDB, black symbols). Panels A and B shows values of the whole body tissues. Panels C, D,

E, F shows the values of the organs: the remaining tissues Re (including the mantle, gonad, digestive gland and labial palps), the adductor muscle Mu and the gills Gi. In panels, C, E grey symbols shows the variation of $\delta^{13}C$ values for gills and remaining tissues that were for lipid normalised according to Post et al.(2007). The vertical bars indicate ±SD of the mean for $n = 5$ oysters.

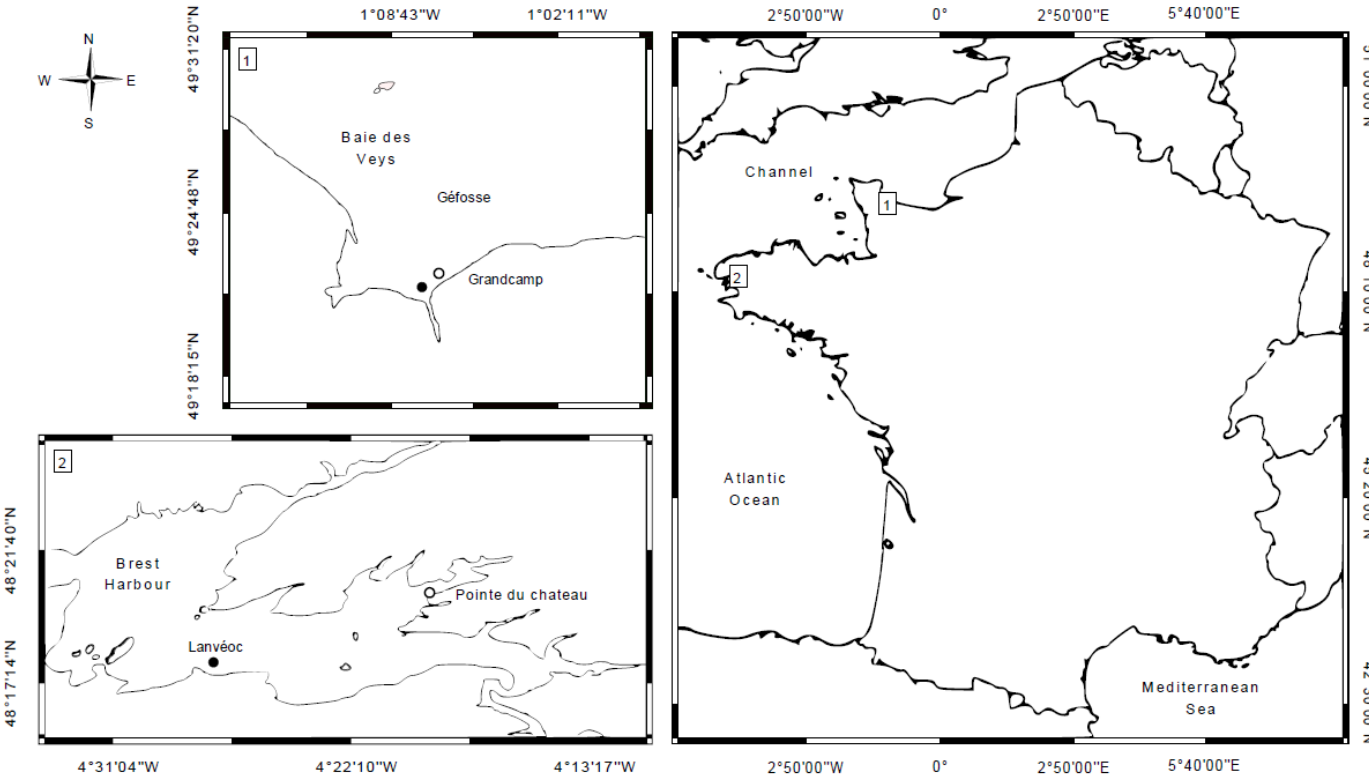
Figure 5: Mean ±SD of isotopic discrimination for $\delta^{13}C$ ($\Delta\delta^{13}C$) and for $\delta^{15}N$ values ($\Delta\delta^{15}N$) between organs (the remaining tissues Re, the adductor muscle Mu and the gills Gi) at two sites: Baie des Veys in Normandy (BDV, white symbols) and Rade de Brest in North Brittany (RDB, black symbols). For both sites, the reference values(=0) are uncorrected $\delta^{13}C$ values ofRe. A : uncorrected $\delta^{13}C$ values (Re values for BDV were slightly repositionned for visual purpose). B : lipid normalised $\delta^{13}C$ values according to Post et al. (2007). Note that $\delta^{15}N$ for the adductor muscle was not corrected as C/N ratio was below 5.

Figure 6: Temporal variations of $\delta^{13}C$ (‰, graph A) and $\delta^{15}N$ (‰, graph B) values of the suspended particulate matter SPOM from March 2009 to February 2010 at two sites: Baie des Veys in Normandy (BDV, white symbols) and Rade de Brest in North Brittany (RDB, black symbols). The vertical bars indicate ±SD of the mean for two replicate samples.

Figure 7 : Variations in $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) values of *Crassostrea gigas* and the potential food sources at Baie des Veys in Normandy (BDV, white symbols) and Rade de Brest in North Brittany (RDB, black symbols) from March to October 2009. $\delta^{13}C$ (‰) values were lipid normalized (Post et al., 2007). $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) were corrected for trophic discrimination (Dubois et al., 2007). SPOM (Suspended Particulate Organic Matter). MPB

(microphytobenthos). MPB_{epi} (epipelic microphytobenthos). $SPOM_{phy}$ (phytoplankton dominated SPOM). The vertical bars indicate ±SD of the mean for n = 12 (SPOM), n = 7 (MPB). Arrows indicate chronological samples of *C. gigas* tissues.

Fig. 1


N
W
E
S
1°08'43"W
1°02'11"W
49°31'20"N
49°24'48"N
49°18'15"N
1
Baie des
Veys
Géfosse
Grandcamp
2
Brest
Harbour
Pointe du chateau
Lanvéoc
48°21'40"N
48°17'14"N
4°31'04"W
4°22'10"W
4°13'17"W
2°50'00"W
0°
2°50'00"E
5°40'00"E
Channel
1
2
Atlantic
Ocean
Mediterranean
Sea

735

Fig. 2

740

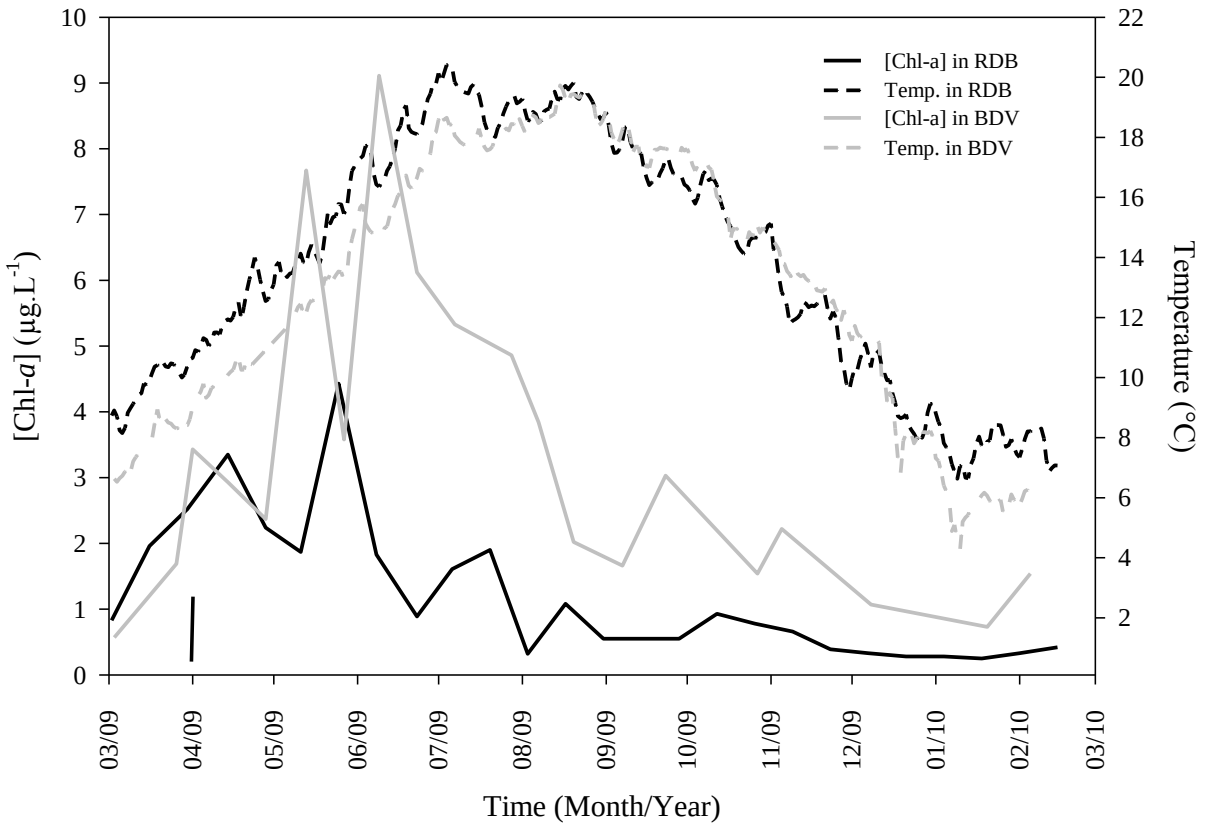
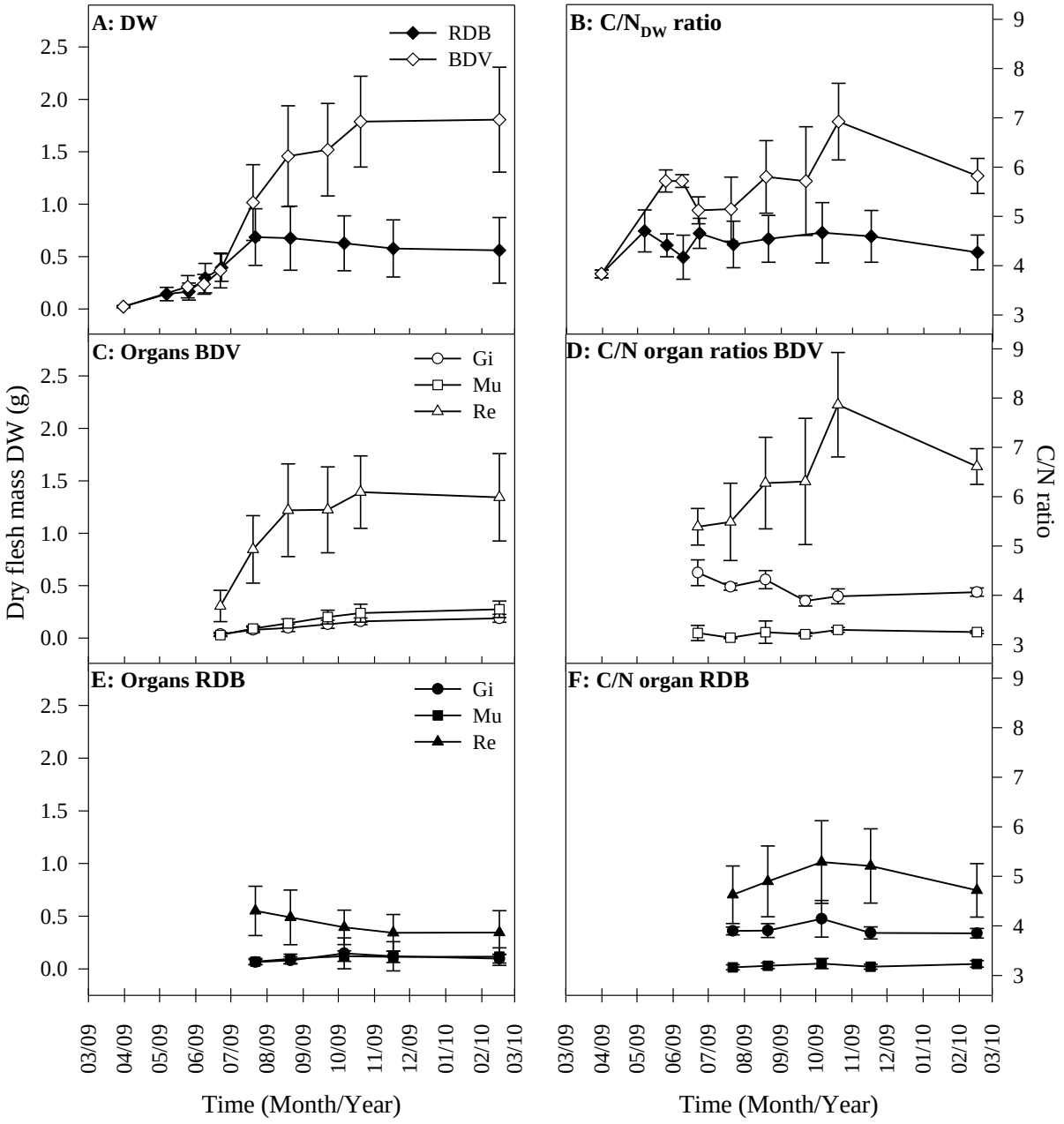
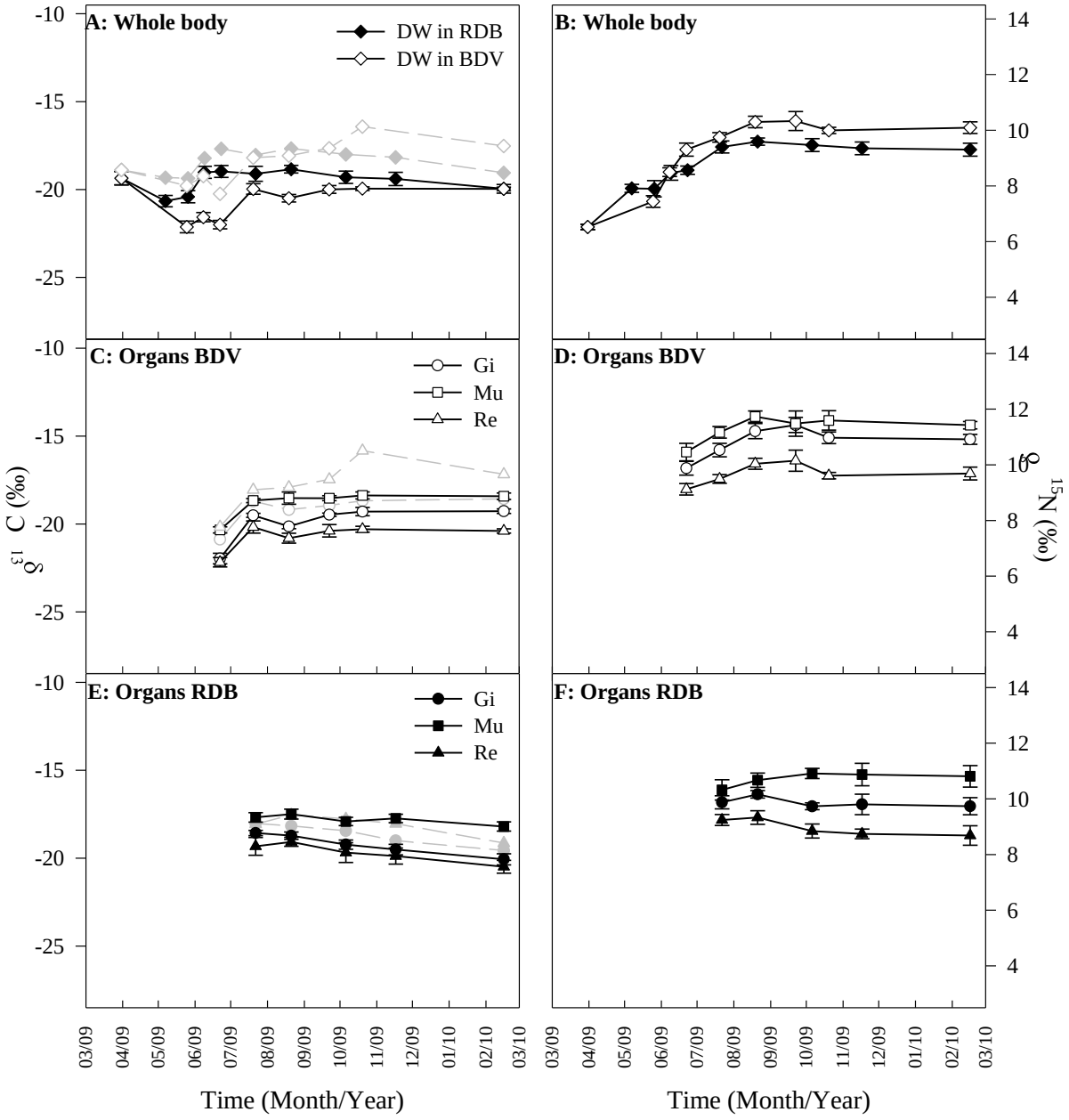

[Chl-a] in RDB
Temp. in RDB
[Chl-a] in BDV
Temp. in BDV
[Chl-a] (µg.L-1)
Temperature (°C)
Time (Month/Year)
03/09
04/09
05/09
06/09
07/09
08/09
09/09
10/09
11/09
12/09
01/10
02/10
03/10

Fig. 3


A: DW
RDB
BDV
B: C/N$_{DW}$ ratio
C: Organs BDV
Gi
Mu
Re
D: C/N organ ratios BDV
E: Organs RDB
F: C/N organ RDB
Dry flesh mass DW (g)
C/N ratio
Time (Month/Year)
03/09
04/09
05/09
06/09
07/09
08/09
09/09
10/09
11/09
12/09
01/10
02/10
03/10

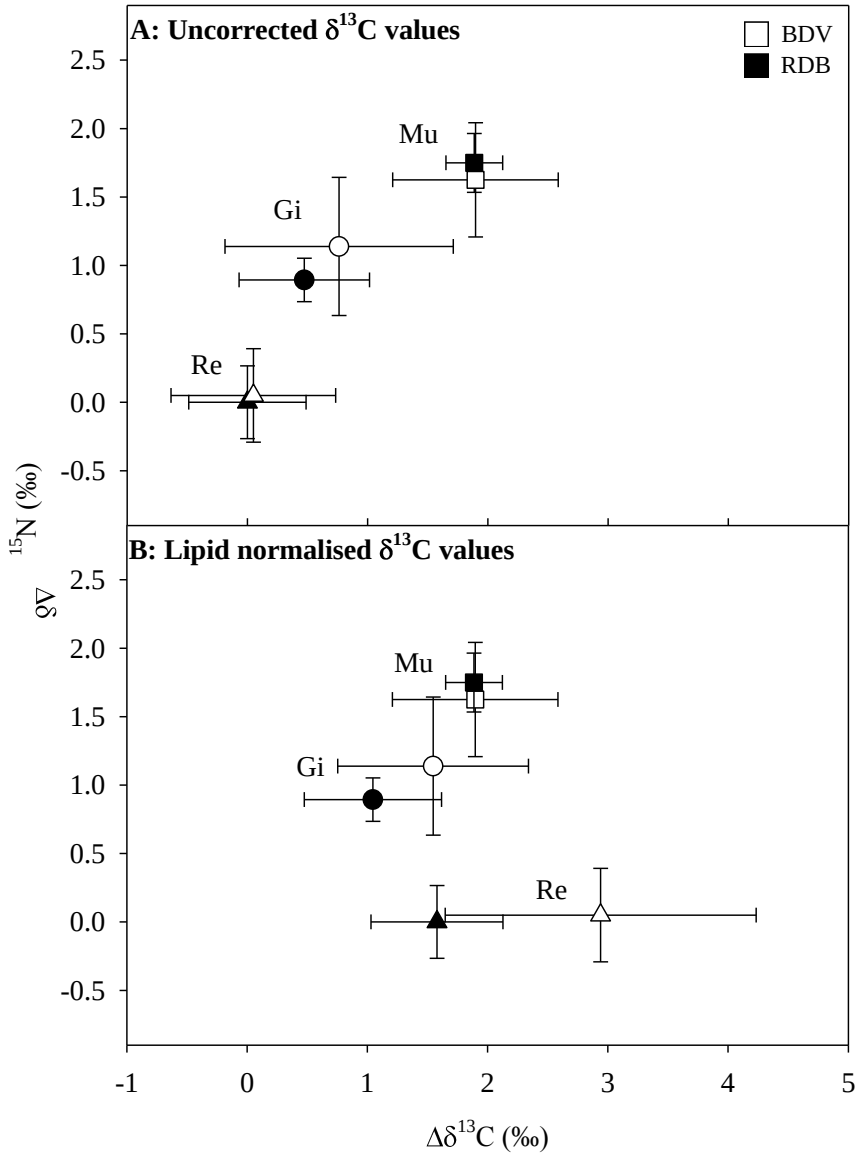
745

Fig. 4


A: Whole body
DW in RDB
DW in BDV
B: Whole body
C: Organs BDV
Gi
Mu
Re
D: Organs BDV
E: Organs RDB
F: Organs RDB
δ13 C (‰)
δ15N (‰)
-10
-15
-20
-25
14
12
10
8
6
4
03/09
04/09
05/09
06/09
07/09
08/09
09/09
10/09
11/09
12/09
01/10
02/10
03/10
Time (Month/Year)
Time (Month/Year)

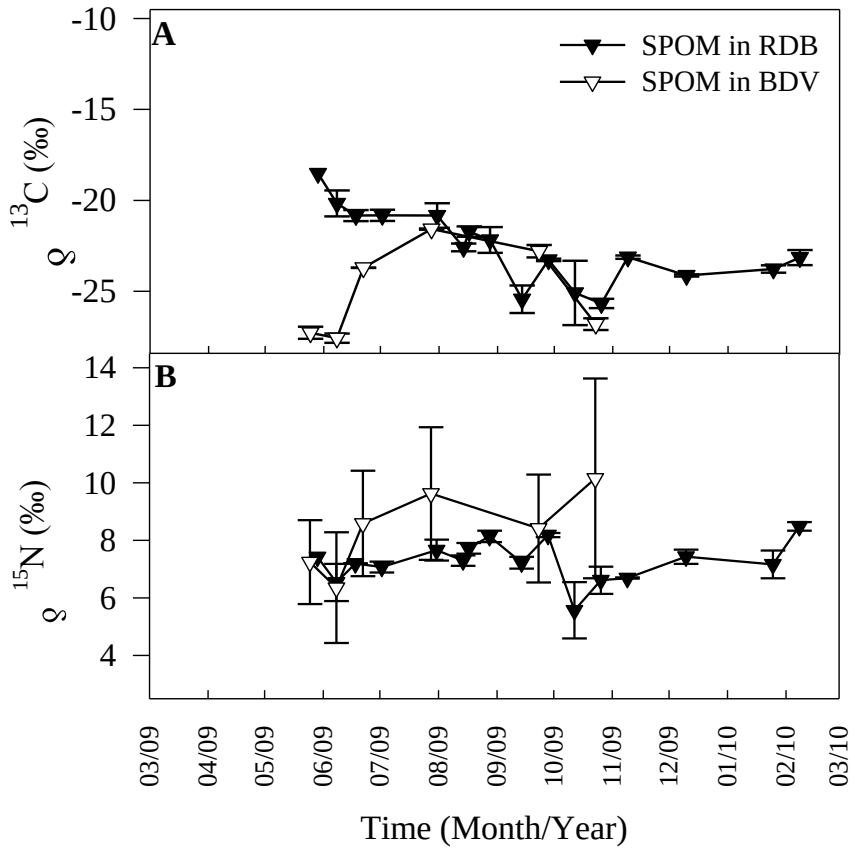
750

Fig. 5


A: Uncorrected δ13C values
BDV
RDB
Mu
Gi
Re
B: Lipid normalised δ13C values
Mu
Gi
Re
δ15N (‰)
Δδ13C (‰)
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1
0
1
2
3
4
5

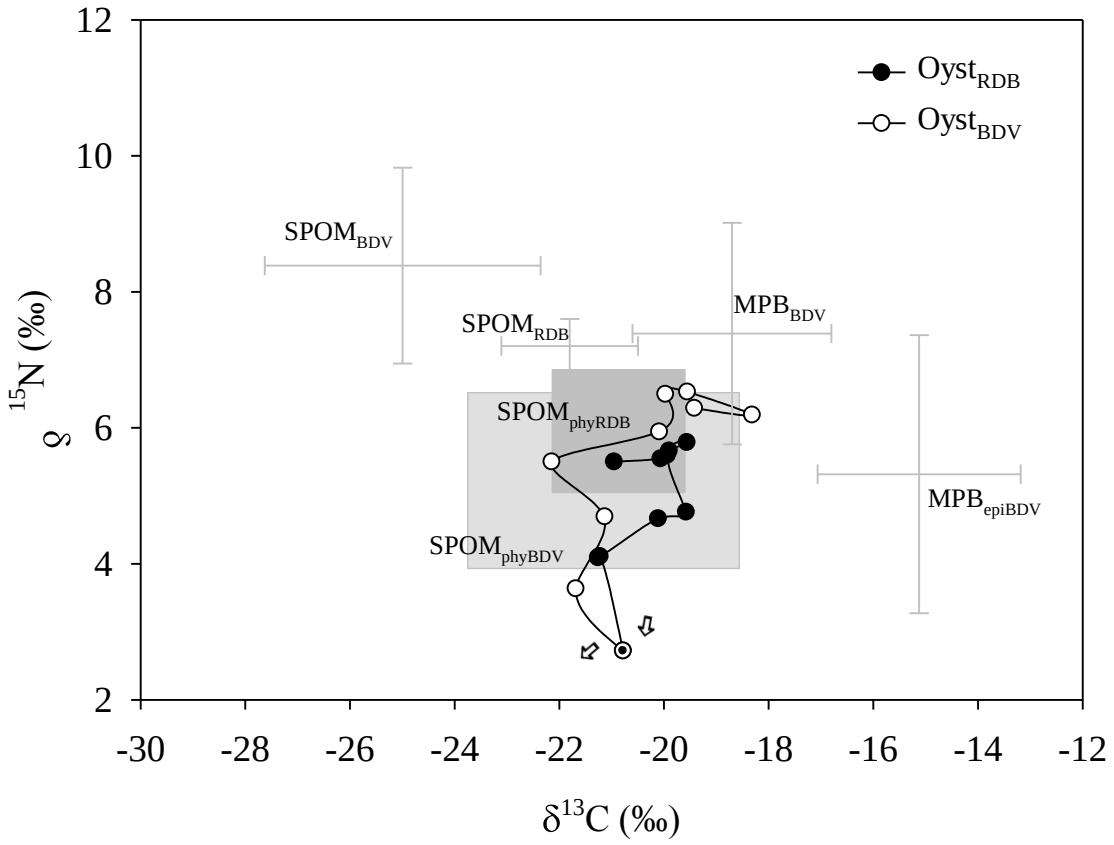
755

Fig. 6


A
B
SPOM in RDB
SPOM in BDV
δ13C (‰)
δ15N (‰)
-10
-15
-20
-25
14
12
10
8
6
4
03/09
04/09
05/09
06/09
07/09
08/09
09/09
10/09
11/09
12/09
01/10
02/10
03/10
Time (Month/Year)

760

Fig 7


12
10
8
6
4
2
-30
-28
-26
-24
-22
-20
-18
-16
-14
-12
δ15N (‰)
δ13C (‰)
Oyst_RDB
Oyst_BDV
SPOM_BDV
SPOM_RDB
MPB_BDV
SPOM_phyRDB
MPB_epiBDV
SPOM_phyBDV